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Barbet , et al. **February 15, 2000**

Nucleic acid vaccines against rickettsial diseases and methods of use

Abstract

Described are nucleic acid vaccines containing MAP1-related genes to protect animals or humans against rickettsial diseases.

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Goverment Interests

This invention was made with government support under ***USAID*** Grant No. LAG-1328-G-00-3030-00. The government has certain rights in this invention .

Claims

We claim:

1. A composition comprising an isolated polynucleotide which encodes a polypeptide having the characteristic of eliciting an immune response protective against disease or death caused by a rickettsial pathogen wherein said polynucleotide has the nucleic acid sequence shown in SEQ ID NO. 1.

2. The composition, according to claim 1, wherein said polynucleotide is operably linked to a vector suitable for use in vaccination.

3. The composition, according to claim 1, further comprising a pharmaceutically acceptable carrier.

Description

DESCRIPTION

TECHNICAL FIELD

This invention relates to nucleic acid vaccines for rickettsial diseases of animals, including humans.

BACKGROUND OF THE INVENTION

The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus, Rickettsia prowazekii, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, e.g., Rickettsia rickettsii and Rickettsia conorii, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe Ehrlichiae have been described. Over 400 cases of human ehrlichiosis, including some fatalities, caused by Ehrlichia chaffeensis have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

Heartwater is another infectious disease caused by a rickettsial pathogen, namely Cowdria ruminantium, and is transmitted by ticks of the genus Amblyomma. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America.

In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur

where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenicol treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] J. Infect. Dis. 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant R. conorii protein were partially protected even against R. ricketsii (Vishwanath, S., G. McDonald, N. Watkins [1990] Infect. Immun. 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes, e.g., protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] Advances in Vet. Sci. and Comp. Med. 27:427-480; Du Plessis, Plessis, J. L. [1970] Onderstepoort J. Vet. Res. 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against R. prowazekii and R. rickettsii. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective," In Rickettsia and Rickettsial Diseases, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood. This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinates. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsia The genes encoding the antigens and that can be employed to recombinantly produce those antigen have also been identified and

sequenced. Certain protective antigens identified for R. rickettsii, R. conorii, and R. prowazekii (e.g. rOmpA and rOmpB) are large (>100 kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue, WHO meeting on nucleic acid vaccines [1994] Vaccine 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CIL), T-helper 1, and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donelly, S. Parker et al. [1993] Science 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specific CTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the Plasmodium yoelii circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] Proc. Natl. Acad. Sci. USA 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox, G., T. Zamb, L. Babiuk [1993] J. Virol 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

BRIEF SUMMARY OF THE INVENTION

Disclosed and claimed here is a novel vaccine for conferring immunity to rickettsia infection, including Cowdria ruminantium causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to of confer immunity in a susceptible host.

The subject invention concerns a nucleic acid, e.g., DNA or mRNA, vaccine containing the major antigenic protein 1 gene (MAP 1) of rickettsial pathogens driven by the human cytomegalovirus (HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention, up to 75% of the immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in in vitro lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia Accordingly, the subject invention concerns the discovery that the gene encoding the MAP1 protein can induce protective immunity as a nucleic acid vaccine against rickettsial disease or death resulting therefrom.

BRIEF DESCRIPTION OF THE DRAWING

FIGS. 1A-1C show a comparison of the amino acid sequences from alignment of the three

rickettsial proteins, namely, Cowdria ruminantium (Cr.), Ehrlichia chaffeensis (E.c.), and Anaplasma marginale (A.m.). (SEQ ID NOS: 1,3, and 5.)

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO. 1 is the coding sequence of the MAP1 gene from Cowdria ruminantium (Highway isolate).

SEQ ID NO. 2 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1.

SEQ ID NO. 3 is the coding sequence of the MAP1 gene from Ehrlichia chaffeensis.

SEQ ID NO. 4 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

SEQ ID NO. 5 is the Anaplasma marginale MSP4 gene coding sequence.

SEQ ID NO. 6 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

DETAILED DISCLOSURE OF THE INVENTION

The subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response. According to the subject invention, recombinant plasmid DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where the antigen is expressed and an immune response induced. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal with gene expression directed for as long as 19 months post-injection (Wolff, J. A., J. J. Ludike, G. Acsadi, P. Williams, A. Jani [1992] Hum. Mol. Genet. 1:363). A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

As described, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been shown to be protective against Cowdria ruminantium, the causative agent of heartwater in domestic ruminants. Accordingly, DNA sequences of rickettsial genes, e.g., MAP1 or homologues thereof, can be useful as nucleic acid vaccines against human and animal rickettsial diseases. The MAP1 gene used to obtain this protection is also present in other rickettsiae including Anaplasma marginale, Ehrlichia canis, and in a causative agent of human ehrlichiosis, Ehrlichia chaffeensis (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] Infect. Immun 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain Rickettsia spp. The MAP1-like gene from Ehrlichia chaffeensis has now been cloned and sequenced.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid

vaccine vectors (plasmids), which are commercially available (eg., Vical, San Diego, Calif.). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E. W. Martin's Remington's Phannaceutical Science, Mack Publishing Company, Easton, Pa.

In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus (HCMV) enhancer-promoter injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50 .mu.l/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCL1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and C. ruminantium antigens in in vitro lymphocyte proliferation tests. These proliferating cells from mice immunized with VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100 .mu.g/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of C. ruminantium. Survival rates of 0% to 3% (1/144 survivors/total in all control groups) were recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, the subject invention concerns the discovery that the gene encoding the MAP1 protein can induce protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses, as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, e.g., whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, Bal31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis et al (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. See also Wei et al. (1983) J. Biol. Chem. 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia Cowdria ruminantium. The vaccine construct tested was the MAP1 gene of C. ruminantium inserted into plasmid VCL1010 (Vical, San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 .mu.g VCL1010/MAP1 DNA (V/M in Table 1 below), or 100, 50 .mu.g VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of C. ruminantium and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

TABLE 1

100 .mu.g V/M	75 .mu.g V/M	50 .mu.g V/M	25 .mu.g V/M	100 .mu.g V	50 .mu.g V	Sal.
Survived						
5	7	5	3	0	0	0
Died 3	1	3	5	8	8	8

The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 .mu.g VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection, 30 mice/group were challenged with 30LD50 of C. ruminantium and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

TABLE 2

V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.

Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls ($p < 0.05$)

Again, as summarized in Table 2, the VCL1010/MAP1 DNA vaccine increased th numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to C. ruminantium in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

EXAMPLE 2

The MAP1 protein of C. ruminantium has significant similarity to MSP4 of A. marginale, and related molecules may also be presenting other rickettsial pathogens. To prove this, we used primers based on regions conserved between C. ruminantium and A. marginale in PCR to clone a MAP1-like gene from E. chaffeensis. The amino acid sequence derived from the cloned E. chaffeensis MAP1-like gene, and alignment with the corresponding genes of C. ruminantium and A. marginale is shown in FIG. 1. We have now identified the regions of MAP1-like genes which are highly conserved between Ehrlichia, Cowdria, and Anaplasma and which can allow cloning of the analogous genes from other rickettsiae.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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- (1) GENERAL INFORMATION:
-    (iii) NUMBER OF SEQUENCES: 6
- (2) INFORMATION FOR SEQ ID NO:1:
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#pairs    (A) LENGTH: 864 base
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: DNA (genomic)
-     (ix) FEATURE:
          (A) NAME/KEY: CDS
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#                 15
- TCA TTT TTA CCT GGT GTG TCC TTT TCT GAT GT - #A ATA CAG GAA GAC AGC
  96
Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Va - #l Ile Gln Glu Asp Ser
#             30
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 144
Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Ly - #s Tyr Met Pro Thr Ala
#         45
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 240
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 288
Asp Ser Ser Asn Thr Asn Ser Thr Ile Phe Th - #r Glu Lys Asp Tyr Ser
#                 95
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Phe Arg Tyr Glu Asn Asn Pro Phe Leu Gly Ph - #e Ala Gly Ala Ile Gly
#           110
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 384
Tyr Ser Met Asn Gly Pro Arg Ile Glu Phe Gl - #u Val Ser Tyr Glu Thr
#       125
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 432
Phe Asp Val Lys Asn Leu Gly Gly Asn Tyr Ly - #s Asn Asn Ala His Met
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- TAC TGT GCT TTA GAT ACA GCA GCA CAA AAT AG - #C ACT AAT GGC GCA GGA
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Tyr Cys Ala Leu Asp Thr Ala Ala Gln Asn Se - #r Thr Asn Gly Ala Gly
145                 1 - #50                 1 - #55                 1 -
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 672
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#40
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Arg Val Ile Gly Asn Glu Phe Lys Asp Ile Al - #a Thr Leu Lys Ile Phe
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 86 - #1
Asp Val Cys His Phe Gly Ile Glu Ile Gly Gl - #y Arg Phe Val Phe
#       285
#            864
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#acids    (A) LENGTH: 287 amino
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: protein
-     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
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#             30
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#         45
- Ser His Phe Gly Lys Met Ser Ile Lys Glu As - #p Ser Lys Asn Thr Gln
#     60
- Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gl - #y Val Lys Thr Pro Ser
# 80
- Asp Ser Ser Asn Thr Asn Ser Thr Ile Phe Th - #r Glu Lys Asp Tyr Ser
#                 95
- Phe Arg Tyr Glu Asn Asn Pro Phe Leu Gly Ph - #e Ala Gly Ala Ile Gly
#           110
- Tyr Ser Met Asn Gly Pro Arg Ile Glu Phe Gl - #u Val Ser Tyr Glu Thr
#       125
- Phe Asp Val Lys Asn Leu Gly Gly Asn Tyr Ly - #s Asn Asn Ala His Met
#   140
- Tyr Cys Ala Leu Asp Thr Ala Ala Gln Asn Se - #r Thr Asn Gly Ala Gly
145                 1 - #50                 1 - #55                 1 -
#60
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#               175
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#           190
- Ser Pro Tyr Val Cys Ala Gly Ile Gly Thr As - #p Leu Val Ser Val Ile
#       205
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225                 2 - #30                 2 - #35                 2 -
#40
- Arg Val Ile Gly Asn Glu Phe Lys Asp Ile Al - #a Thr Leu Lys Ile Phe
#               255
- Thr Ser Lys Thr Gly Ile Ser Asn Pro Gly Ph - #e Ala Ser Ala Thr Leu
#           270
- Asp Val Cys His Phe Gly Ile Glu Ile Gly Gl - #y Arg Phe Val Phe
#       285
- (2) INFORMATION FOR SEQ ID NO:3:
-      (i) SEQUENCE CHARACTERISTICS:
#pairs    (A) LENGTH: 842 base
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: DNA (genomic)
-     (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 1..840
-     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
- ATG AAT TAC AAA AAA AGT TTC ATA ACA GCG AT - #T GAT ATC ATT AAT ATC
  48
Met Asn Tyr Lys Lys Ser Phe Ile Thr Ala Il - #e Asp Ile Ile Asn Ile
#       300
- CTT CTC TTA CCT GGA GTA TCA TTT TCC GAC CC - #A AGG CAG GTA GTG GTC
  96
Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pr - #o Arg Gln Val Val Val
#   315
- ATT AAC GGT AAT TTC TAC ATC AGT GGA AAA TA - #C GAT GCC AAG GCT TCG
 144
Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Ty - #r Asp Ala Lys Ala Ser
320                 3 - #25                 3 - #30                 3 -
#35
- CAT TTT GGA GTA TTC TCT GCT AAG GAA GAA AG - #A AAT ACA ACA GTT GGA
 192
His Phe Gly Val Phe Ser Ala Lys Glu Glu Ar - #g Asn Thr Thr Val Gly
#               350
- GTG TTT GGA CTG AAG CAA AAT TGG GAC GGA AG - #C GCA ATA TCC AAC TCC
 240
Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Se - #r Ala Ile Ser Asn Ser
#           365
- TCC CCA AAC GAT GTA TTC ACT GTC TCA AAT TA - #T TCA TTT AAA TAT GAA
 288
Ser Pro Asn Asp Val Phe Thr Val Ser Asn Ty - #r Ser Phe Lys Tyr Glu
#       380
- AAC AAC CCG TTT TTA GGT TTT GCA GGA GCT AT - #T GGT TAC TCA ATG GAT
 336
Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Il - #e Gly Tyr Ser Met Asp
#   395
- GGT CCA AGA ATA GAG CTT GAA GTA TCT TAT GA - #A ACA TTT GAT GTA AAA
 384
Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Gl - #u Thr Phe Asp Val Lys
400                 4 - #05                 4 - #10                 4 -
#15
- AAT CAA GGT AAC AAT TAT AAG AAT GAA GCA CA - #T AGA TAT TGT GCT CTA
 432
Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala Hi - #s Arg Tyr Cys Ala Leu
#               430
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- TCC CAT AAC TCA GCA GCA GAC ATG AGT AGT GC - #A AGT AAT AAT TTT GTC
 480
Ser His Asn Ser Ala Ala Asp Met Ser Ser Al - #a Ser Asn Asn Phe Val
#           445
- TTT CTA AAA AAT GAA GGA TTA CTT GAC ATA TC - #A TTT ATG CTG AAC GCA
 528
Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Se - #r Phe Met Leu Asn Ala
#       460
- TGC TAT GAC GTA GTA GGC GAA GGC ATA CCT TT - #T TCT CCT TAT ATA TGC
 576
Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Ph - #e Ser Pro Tyr Ile Cys
#   475
- GCA GGT ATC GGT ACT GAT TTA GTA TCC ATG TT - #T GAA GCT ACA AAT CCT
 624
Ala Gly Ile Gly Thr Asp Leu Val Ser Met Ph - #e Glu Ala Thr Asn Pro
480                 4 - #85                 4 - #90                 4 -
#95
- AAA ATT TCT TAC CAA GGA AAG TTA GGT TTA AG - #C TAC TCT ATA AGC CCA
 672
Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Se - #r Tyr Ser Ile Ser Pro
#               510
- GAA GCT TCT GTG TTT ATT GGT GGG CAC TTT CA - #T AAG GTA ATA GGG AAC
 720
Glu Ala Ser Val Phe Ile Gly Gly His Phe Hi - #s Lys Val Ile Gly Asn
#           525
- GAA TTT AGA GAT ATT CCT ACT ATA ATA CCT AC - #T GGA TCA ACA CTT GCA
 768
Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Th - #r Gly Ser Thr Leu Ala
#       540
- GGA AAA GGA AAC TAC CCT GCA ATA GTA ATA CT - #G GAT GTA TGC CAC TTT
 816
Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Le - #u Asp Val Cys His Phe
#   555
#             842  GA GGA AGG TTT AA
Gly Ile Glu Met Gly Gly Arg Phe
560                 5 - #65
- (2) INFORMATION FOR SEQ ID NO:4:
-      (i) SEQUENCE CHARACTERISTICS:
#acids    (A) LENGTH: 280 amino
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: protein
-     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
- Met Asn Tyr Lys Lys Ser Phe Ile Thr Ala Il - #e Asp Ile Ile Asn Ile
#                 15
- Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pr - #o Arg Gln Val Val Val
#             30
- Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Ty - #r Asp Ala Lys Ala Ser
#         45
- His Phe Gly Val Phe Ser Ala Lys Glu Glu Ar - #g Asn Thr Thr Val Gly
#     60
- Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Se - #r Ala Ile Ser Asn Ser
# 80
- Ser Pro Asn Asp Val Phe Thr Val Ser Asn Ty - #r Ser Phe Lys Tyr Glu
#                 95
- Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Il - #e Gly Tyr Ser Met Asp
#           110
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- Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Gl - #u Thr Phe Asp Val Lys
#       125
- Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala Hi - #s Arg Tyr Cys Ala Leu
#   140
- Ser His Asn Ser Ala Ala Asp Met Ser Ser Al - #a Ser Asn Asn Phe Val
145                 1 - #50                 1 - #55                 1 -
#60
- Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Se - #r Phe Met Leu Asn Ala
#               175
- Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Ph - #e Ser Pro Tyr Ile Cys
#           190
- Ala Gly Ile Gly Thr Asp Leu Val Ser Met Ph - #e Glu Ala Thr Asn Pro
#       205
- Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Se - #r Tyr Ser Ile Ser Pro

#   220
- Glu Ala Ser Val Phe Ile Gly Gly His Phe Hi - #s Lys Val Ile Gly Asn
225                 2 - #30                 2 - #35                 2 -
#40
- Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Th - #r Gly Ser Thr Leu Ala
#               255
- Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Le - #u Asp Val Cys His Phe
#           270
- Gly Ile Glu Met Gly Gly Arg Phe
#       280
- (2) INFORMATION FOR SEQ ID NO:5:
-      (i) SEQUENCE CHARACTERISTICS:
#pairs    (A) LENGTH: 849 base
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: DNA (genomic)
-     (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 1..846
-     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
- ATG AAT TAC AGA GAA TTG TTT ACA GGG GGC CT - #G TCA GCA GCC ACA GTC
  48
Met Asn Tyr Arg Glu Leu Phe Thr Gly Gly Le - #u Ser Ala Ala Thr Val
#               295
- TGC GCC TGC TCC CTA CTT GTT AGT GGG GCC GT - #A GTG GCA TCT CCC ATG
  96
Cys Ala Cys Ser Leu Leu Val Ser Gly Ala Va - #l Val Ala Ser Pro Met
#           310
- AGT CAC GAA GTG GCT TCT GAA GGG GGA GTA AT - #G GGA GGT AGC TTT TAC
 144
Ser His Glu Val Ala Ser Glu Gly Gly Val Me - #t Gly Gly Ser Phe Tyr
#       325
- GTG GGT GCG GCC TAC AGC CCA GCA TTT CCT TC - #T GTT ACC TCG TTC GAC
 192
Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Se - #r Val Thr Ser Phe Asp
#   340
- ATG CGT GAG TCA AGC AAA GAG ACC TCA TAC GT - #T AGA GGC TAT GAC AAG
 240
Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Va - #l Arg Gly Tyr Asp Lys
345                 3 - #50                 3 - #55                 3 -
#60
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- AGC ATT GCA ACG ATT GAT GTG AGT GTG CCA GC - #A AAC TTT TCC AAA TCT
 288
Ser Ile Ala Thr Ile Asp Val Ser Val Pro Al - #a Asn Phe Ser Lys Ser
#               375
- GGC TAC ACT TTT GCC TTC TCT AAA AAC TTA AT - #C ACG TCT TTC GAC GGC
 336
Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Il - #e Thr Ser Phe Asp Gly
#            390
- GCT GTG GGA TAT TCT CTG GGA GGA GCC AGA GT - #G GAA TTG GAA GCG AGC
 384
Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Va - #l Glu Leu Glu Ala Ser
#       405
- TAC AGA AGG TTT GCT ACT TTG GCG GAC GGG CA - #G TAC GCA AAA AGT GGT
 432
Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gl - #n Tyr Ala Lys Ser Gly
#   420
- GCG GAA TCT CTG GCA GCT ATT ACC CGC GAC GC - #T AAC ATT ACT GAG ACC
 480
Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Al - #a Asn Ile Thr Glu Thr
425                 4 - #30                 4 - #35                 4 -
#40
- AAT TAC TTC GTA GTC AAA ATT GAT GAA ATC AC - #A AAC ACC TCA GTC ATG
 528
Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Th - #r Asn Thr Ser Val Met
#               455
- TTA AAT GGC TGC TAT GAC GTG CTG CAC ACA GA - #T TTA CCT GTG TCC CCG
 576
Leu Asn Gly Cys Tyr Asp Val Leu His Thr As - #p Leu Pro Val Ser Pro
#           470
- TAT GTA TGT GCC GGG ATA GGC GCA AGC TTT GT - #T GAC ATC TCT AAG CAA
 624
Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Va - #l Asp Ile Ser Lys Gln
#       485
- GTA ACC ACA AAG CTG GCC TAC AGG GGC AAG GT - #T GGG ATT AGC TAC CAG
 672
Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Va - #l Gly Ile Ser Tyr Gln
#   500
- TTT ACT CCG GAA ATA TCC TTG GTG GCA GGT GG - #G TTC TAC CAC GGG CTA
 720
Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gl - #y Phe Tyr His Gly Leu
505                 5 - #10                 5 - #15                 5 -
#20
- TTT GAT GAG TCT TAC AAG GAC ATT CCC GCA CA - #C AAC AGT GTA AAG TTC
 768
Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala Hi - #s Asn Ser Val Lys Phe
#               535
- TCT GGA GAA GCA AAA GCC TCA GTC AAA GCG CA - #T ATT GCT GAC TAC GGC
 816
Ser Gly Glu Ala Lys Ala Ser Val Lys Ala Hi - #s Ile Ala Asp Tyr Gly
#           550
#        849T GGA GCA AGA TTC CTG TTC AGC TA - #A
Phe Asn Leu Gly Ala Arg Phe Leu Phe Ser
#       560
- (2) INFORMATION FOR SEQ ID NO:6:
-      (i) SEQUENCE CHARACTERISTICS:
#acids    (A) LENGTH: 282 amino
          (B) TYPE: amino acid
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          (D) TOPOLOGY: linear
-     (ii) MOLECULE TYPE: protein
-     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Met Asn Tyr Arg Glu Leu Phe Thr Gly Gly Le - #u Ser Ala Ala Thr Val
#                 15
- Cys Ala Cys Ser Leu Leu Val Ser Gly Ala Va - #l Val Ala Ser Pro Met
#             30
- Ser His Glu Val Ala Ser Glu Gly Gly Val Me - #t Gly Gly Ser Phe Tyr
#         45
- Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Se - #r Val Thr Ser Phe Asp
#     60
- Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Va - #l Arg Gly Tyr Asp Lys
# 80
- Ser Ile Ala Thr Ile Asp Val Ser Val Pro Al - #a Asn Phe Ser Lys Ser
#                 95
- Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Il - #e Thr Ser Phe Asp Gly
#           110
- Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Va - #l Glu Leu Glu Ala Ser
#       125
- Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gl - #n Tyr Ala Lys Ser Gly
#   140
- Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Al - #a Asn Ile Thr Glu Thr
145                 1 - #50                 1 - #55                 1 -
#60
- Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Th - #r Asn Thr Ser Val Met
#               175
- Leu Asn Gly Cys Tyr Asp Val Leu His Thr As - #p Leu Pro Val Ser Pro
#           190
- Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Va - #l Asp Ile Ser Lys Gln
#       205
- Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Va - #l Gly Ile Ser Tyr Gln
#   220
- Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gl - #y Phe Tyr His Gly Leu
225                 2 - #30                 2 - #35                 2 -
#40
- Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala Hi - #s Asn Ser Val Lys Phe
#               255
- Ser Gly Glu Ala Lys Ala Ser Val Lys Ala Hi - #s Ile Ala Asp Tyr Gly
#           270
- Phe Asn Leu Gly Ala Arg Phe Leu Phe Ser
#       280
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